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Different Mechanisms of White Matter Abnormalities in ADHD: A DTI Study

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ABSTRACT

BACKGROUND Literature regarding white matter (WM) abnormalities in Attention-Deficit/Hyperactivity Disorder (ADHD) is sparse and inconsistent. In this article, we shed more light on WM microstructure in ADHD, its association with symptom count, and the familiality of WM abnormalities in ADHD.

METHODS Diffusion Tensor Imaging (DTI) was performed in a large sample of individuals with ADHD (n=170), their unaffected siblings (n=80), and healthy controls (n=107), aged 8-30 years. Extensive categorical as well as dimensional data regarding ADHD status and symptom count were collected. A whole-brain voxel-wise approach was used to investigate associations between ADHD status and symptom count and WM microstructure, as measured by fractional anisotropy (FA) and mean diffusivity (MD).

RESULTS Individuals with ADHD showed decreased FA and decreased MD in several widespread, non-overlapping brain regions. In contrast, higher ADHD symptom count was consistently associated with increased FA and decreased MD in the ADHD group. Unaffected siblings resembled the ADHD group with regard to decreased FA, but had similar MD to healthy controls. Results were not confounded by socio-economic status, the presence of comorbidities, or a history of medication use.

CONCLUSION Our results indicate widespread disturbances in WM microstructure in ADHD, which seem to be driven by two different mechanisms. Decreased FA in ADHD may be due to a familial vulnerability to the disorder, while a second mechanism may drive the association between ADHD symptom count and both higher FA and lower MD. Such different mechanisms may play an important role in the inconsistencies found in the current literature.

INTRODUCTION

Neuroimaging studies suggest that alterations in structural and functional brain connectivity might (partly) underlie behavioural symptoms of Attention-Deficit/Hyperactivity Disorder (ADHD; Konrad et al., 2010). Diffusion Tensor Imaging (DTI), a technique often used to measure structural brain connectivity, allows quantification of the microstructural properties of brain white matter (WM) by measuring diffusivity of water molecules. Commonly used DTI parameters include Fractional Anisotropy (FA) and Mean Diffusivity (MD), which rely on the direction and strength of water diffusion respectively (Alexander, Lee, Lazar, & Field, 2007; Beaulieu, 2002). FA is often interpreted as indicative of axonal integrity and organization, whereas MD is generally associated with changes in the inter-cellular space. It should be noted, however, that while diffusion parameters are overall quite sensitive to tissue properties such as axonal orientation, axonal density, and myelination, no DTI measure is specifically sensitive to one property (Jones, Knosche, & Turner, 2013).

Converging evidence from DTI studies in ADHD points towards a widespread pattern of WM disturbances throughout the brain in ADHD (Chuang et al., 2013; Cortese et al., 2013; Van Ewijk et al., 2012). However, the current literature suffers from a great heterogeneity in findings in terms of the location and direction of abnormal FA and MD values. Part of this heterogeneity may be due to differences in sample characteristics (e.g. different age ranges, comorbidities, or diagnostic methods) and research methods (e.g. multiple comparison or head movement correction). However, it is unlikely that such factors could explain why FA and MD values have been found to be both lower and higher in ADHD across different studies. Hence, it is currently difficult, if not impossible, to draw conclusions regarding the location and severity of WM disturbances in ADHD, and to interpret these disturbances in terms of underlying microstructural abnormalities.

Over the past decade, the view on ADHD psychopathology has shifted from a categorical, qualitative disease definition to a more dimensional, quantitative view, in which symptoms lie on a continuum from normal to abnormal behaviour, with ADHD as a clinical disorder on the extreme (Lubke et al., 2009). Consistent with this view, it should be expected that ADHD-related neurobiological abnormalities are also quantitative rather than qualitative in nature, and that they are associated with the number of ADHD symptoms. Hence, studying the brain correlates associated with dimensional measures of ADHD may provide a more powerful approach than examining the neurobiology of categorical distinctions (Morris & Cuthbert,

2012). Studies investigating dimensional associations between ADHD and WM microstructure are sparse and inconsistent. Three studies found correlations between behavioural measures of ADHD and FA or MD values in some of their regions of interest (ROIs; Ashtari et al., 2005; Lawrence et al., 2013; Peterson et al., 2011), suggesting that the behavioural symptoms of ADHD are associated with WM pathology. One of these studies found a negative relationship between FA in the cerebellum and inattention in ADHD patients (Ashtari et al., 2005). A second study found a positive relationship between MD in the forceps minor and inattentive symptoms across the three groups they examined (ADHD, unaffected siblings, and healthy controls; Lawrence et al., 2013), and the third study reported a positive correlation between FA in the sagittal stratum and total ADHD symptomatology across ADHD and healthy controls (Peterson et al., 2011). Notably, these studies differ greatly in location and direction of their findings, and are thus difficult to integrate. Other studies that investigated the association between dimensional measures of ADHD and DTI parameters reported negative results (Ashtari et al., 2005; Dramsdahl et al., 2012; Hamilton et al., 2008; Lawrence et al., 2013). To our knowledge, no studies have adopted a whole-brain voxel-wise approach to explore the association between ADHD symptom count and WM abnormalities throughout the whole brain.

A virtually unexplored issue is the familiality of WM abnormalities in ADHD, which can be investigated by including unaffected siblings. If WM abnormalities reflect a familial vulnerability to the disorder, unaffected siblings are expected to show similar WM abnormalities as their affected siblings - albeit possibly to a lesser extent. Up to date, only one DTI study has included unaffected siblings of individuals with ADHD (Lawrence et al., 2013). The authors found no disturbances in FA in the ADHD group, but MD was elevated in individuals with ADHD as well as their unaffected siblings in the superior longitudinal fasciculus, forceps minor, and anterior thalamic radiation, giving first support for a heritable component in WM abnormalities in ADHD.

The current study aimed to explore microstructural properties of WM brain tissue in children, adolescents, and young adults with ADHD. By using a large, carefully and extensively phenotyped sample, a more robust image of WM pathology in ADHD should emerge. Inclusion of unaffected siblings allowed us to investigate the familiality of WM abnormalities in ADHD. Additionally, a whole-brain analysis using a dimensional approach was used to investigate whether inattentive and hyperactive-impulsive symptoms were associated with

WM abnormalities in ADHD. Based on the current literature, widespread WM abnormalities in ADHD were expected to be found. However, due to the high heterogeneity of previous findings (Van Ewijk et al., 2012), no specific hypotheses were formed regarding the location and direction of expected effects.

METHODS

Participants

For a full description, see previous work (Van Ewijk et al., 2014). In short, participants originally took part in the Dutch part of the International Multicenter ADHD Genetics (IMAGE) study (Muller et al., 2011), and were re-invited for the current follow-up study between 2009 and 2012 (NeuroIMAGE study; also see www.neuroimage.nl). Inclusion criteria were: age between 5-30 years, European Caucasian descent, IQ≥70, and no epilepsy, neurological disorder, or known genetic disorder (such as Fragile X syndrome or Down syndrome). Comorbid psychiatric disorders reported by parents were excluded, except for oppositional defiant disorder (ODD), conduct disorder (CD), and pervasive developmental disorder not otherwise specified (PDD-NOS), given their high co-occurrence in ADHD. Complete data were available of 357 participants who met inclusion criteria, including 170 ADHD patients (96 combined, 63 predominantly inattentive, and 11 predominantly hyperactive-impulsive type), 80 unaffected siblings of ADHD patients, and 107 healthy controls. Note that all subjects were required to meet criteria for the same diagnostic group at entry to the cohort, and consequently all individuals with ADHD represent cases with persistent ADHD.

Diagnostic Assessment

For a full description, see previous work (Van Ewijk et al., 2014). In short, to determine ADHD diagnoses at follow-up, all participants were assessed with a semi-structured diagnostic interview Schedule for Affective Disorders and Schizophrenia for School-Age Children - Present and Lifetime Version (K-SADS-PL; Kaufman et al., 1997) and Conners' ADHD questionnaires from multiple informants (Conners, Erhardt, & Sparrow, 1999; Conners, Sitarenios, Parker, & Epstein, 1998a, 1998b). An algorithm was applied to create a combined symptom count from the interview and questionnaires. Participants were diagnosed with ADHD when they met full DSM-IV (American Psychiatric Association, 2000) criteria for the disorder. All unaffected

participants - unaffected siblings and controls alike - were required to score ≤ three symptoms on both symptom dimensions, to ensure they did not show signs of (subthreshold) ADHD. Criteria were slightly adapted for young adults (≥18 years), such that a combined symptom count of five symptoms was sufficient for a diagnosis (Kooij et al., 2005), and ≤ two symptoms on both symptom dimensions were required for an unaffected status. For dimensional analyses, the sum of the symptom counts on both symptom dimensions was used. The presence of ODD and CD was evaluated using two additional K-SADS-PL sections. The presence of subclinical autism spectrum disorder (ASD) symptoms was evaluated by the Children's Social Behavior Questionnaire (CSBQ). Additional information was collected from custom questionnaires, in which parents indicated whether their child had received medication for ADHD (current or past) and how many years of education both parents had received.

Procedure

The current study was part of a comprehensive assessment protocol, including a DTI scan. IQ was estimated by the Vocabulary and Block Design subtests of the Wechsler Intelligence Scale for Children-III (WISC-III) or Wechsler Adult Intelligence Scale III (WAIS-III; for participants ≥17 years). Data acquisition was carried out in the Netherlands. Participants were asked to withhold the use of psychoactive medication for 48 hours before measurement. Fifteen participants were not able to comply, resulting in nine participants with a 24-hour washout (6 using stimulants, 1 antipsychotics, 1 opioid and 1 atomoxetine) and six participants using medication during measurement (2 on stimulants, 1 antipsychotics, 1 antidepressants, and 2 with a combination of these medications). All participants were familiarized with the scanning procedure using a mock-scanner. The study was approved by the Dutch local medical ethics committees. Informed consent was signed by all participants (parents signed informed consent for participants under 12 years of age). Afterwards, participants received a reward of €50 and a copy of their MRI scan.

Imaging acquisition and (pre-)processing

MRI scanning was carried out on either a 1.5 Tesla Sonata or a 1.5 Tesla Avanto MRI scanner (Siemens, Erlangen, Germany), using the same Siemens 8-channel head coil. Whole-brain, high-resolution T1-weighted anatomical images were acquired in the sagittal plane (MP-RAGE, 176 slices, acquisition matrix 256x256, voxel size 1x1x1mm; TE/TR=2.95/2730ms, TI=1000ms, FA=7°, GRAPPA-acceleration 2). Eddy-current compensated diffusion-weighted

SE-EPI images were collected during one acquisition consisting of five volumes without directional weighting (b value of zero), followed by 60 volumes with non-collinear gradient directions (60 interleaved slices, matrix 64x64, voxel size 2x2x2.2mm, TE/TR=97/8500ms, b-value 1000s/mm^2, GRAPPA-acceleration 2).

SPM8 (Wellcome Trust Centre for Neuroimaging) functionality was employed to correct for residual eddy currents and realign all diffusion-weighted images of each subject using affine transformations and mutual information as a cost function. Mutual information was also used to first rigidly co-register the realigned images with the T1 image and then non-linearly along the EPI phase-encode direction to unwarp the imaging distortions that result from magnetic susceptibility inhomogeneities (Visser, Qin, & Zwiers, 2010). The PATCH method (Zwiers, 2010) was applied to correct for motion-induced artefacts and robustly estimate the diffusion tensor images. From these tensor images, 3D FA and MD maps were derived for each subject, and further processed using the standard procedures within the Tract Based Spatial Statistics (TBSS; Smith et al., 2006) toolbox of FSL (FMRIB Analysis group, Oxford, UK). Each participant's DTI scan and FA map were manually screened for the presence of scan artefacts, such as problems caused by unwarping the images, motion-induced artefacts, and insufficient coverage of the scanning field. If artefacts could not be removed, this participant was excluded from subsequent analyses (n=7). Subsequently, all FA and MD maps were brain-extracted using the BET tool, and non-linearly transformed to MNI152 standard space and averaged into a single 3D image, on which a mean WM skeleton was created. A threshold was set (FA>.20) in order to reduce cross-participant variability and misalignment and to restrict analyses to the main WM tracts. Finally, each participant's FA and MD image was projected onto this skeleton, and resulting data were used for voxelwise statistics.

Data analysis

Group differences in sample characteristics were investigated using analysis of variance and chi square tests in SPSS (version 21, IBM, Chicago, IL, USA). Voxelwise analyses were performed in FSL to examine whole-brain group differences on the white matter skeleton. The randomise tool in FSL was used, which is based on permutation testing. Results were obtained using Threshold-Free Cluster Enhancement (TFCE; Smith & Nichols, 2009), providing results at $p<.05$ using FWE correction for multiple testing. Anatomical labels of voxels showing significant effects were identified using the built-in JHU atlases for WM tracts in FSLview and the MRI Atlas of Human White Matter (Mori, Wakana, Nagae-Poetscher, & Van Zijl, 2005).

Two types of TBSS analyses were performed. First, to examine categorical group differences between ADHD patients and healthy controls, a General Linear Model was built with 'group' as predictor. Second, to investigate whether WM disturbances in ADHD would be associated with symptom count, a dimensional analysis was performed within the ADHD group, using the combined symptom count as predictor. Given the unequal distribution of groups over the two scan sites, and group differences in gender and IQ (see Table 1), these factors were added to both models as covariates. Although there were no significant group differences in age, we added age as a covariate given the broad age range of our sample and the important impact of age on white matter organization in the brain (Asato, Terwilliger, Woo, & Luna, 2010).

Subsequently, several complementary analyses were conducted, in which the significant results from the TBSS analyses were further investigated. As such, these analyses do not represent full-brain analyses but only apply to the clusters of significant results found in the TBSS analyses. Complementary analyses were set up for several purposes: to investigate WM microstructure in unaffected siblings, to explore the possible differential effect of both ADHD symptom dimensions, to investigate whether the broad age range of our sample confounded our main results, and to investigate the effect of other possible confounders. All complementary analyses were conducted as ROI analyses to increase power. ROI masks were created for clusters that showed significant group differences in the categorical TBSS analysis ('categorical ROI'), and for clusters that showed a significant association in the dimensional TBSS analysis ('dimensional ROI'). Subsequently, for each participant, mean FA and MD values were extracted from both these ROIs, to be used as dependent variables. Linear Mixed Models (SPSS) were built with a random intercept per family to account for correlated data within families. The same covariates were used as in the TBSS analyses (i.e. scan site, age, gender and IQ), and post-hoc analyses were performed using Fisher's least significant difference (LSD). First, to investigate WM microstructure in unaffected siblings, a model was built with group (ADHD, unaffected siblings, and controls) as a predictor for FA and MD in the categorical ROI. Interactions between the group predictor and age, gender and IQ were initially included in the model, but were dropped if they proved non-significant. To test whether socio-economic status (SES) confounded our results, this variable was added to the model to examine if the relationship between group and FA and MD changed. SES was reflected by the completed education level of the parents, recoded into a measure reflecting years of education (Buis, 2010), averaged over both parents. Second, to investigate the possible differential influence

of the two ADHD symptom dimensions (inattentive versus hyperactive-impulsive symptoms) on WM abnormalities, a model was built with symptom count on both dimensions as two separate predictors for FA and MD in the dimensional ROI (ADHD group only). Both predictors were added to the model simultaneously and were subsequently removed one at a time to examine changes in the model. We examined possible interactions and confounders in a similar manner as in the categorical model, and tested three additional confounders: presence of ODD/CD, autism spectrum symptom score, and history of medication use (yes/no). To test whether our main results applied to the full age range of our sample, we re-ran our main analyses in three separate age groups (see Supplement 1).

RESULTS

Sample characteristics are summarized in Table 1.

The categorical TBSS analysis revealed a relatively widespread pattern of reduced FA (bilateral) and MD (right hemisphere) in the ADHD group, compared to healthy controls (Figure 1A, 1B, Supplementary Table S1). Locations of clusters of reduced FA and MD were not overlapping. No clusters of elevated FA or MD were observed in ADHD. The dimensional TBSS analysis revealed a widespread pattern of associations between the combined symptom count and both FA and MD within the ADHD group (Figure 1C, 1D, Supplementary Table S1). A higher number of ADHD symptoms were significantly associated with higher FA and with lower MD. Of note, while MD was consistently lower in more severe cases of ADHD, as was expected from the categorical analysis, FA findings were higher in more severe cases, thus being in the opposite direction of what was expected from the categorical analysis. While a large portion of the dimensional findings was located in different regions than the categorical findings, some overlap was present. For both FA and MD, in about 25% of the voxels in which a group difference was found, a significant association with ADHD symptom count was also found (see Figure 2 for FA and MD values in these overlapping regions).

Subsequent complementary analyses were conducted on mean FA and MD values, extracted from significant clusters in the TBSS analyses. A complementary analysis was conducted to examine the familiality of ADHD-related WM disturbances in the categorical ROI, comparing ADHD patients, unaffected siblings, and controls (results shown in Table 2). A significant main effect of group on FA as well as MD was found, but no significant interactions between group

Table 1. Sample characteristics.

	ADHD (n=170)	Unaffected siblings (n=80)	Controls (n=107)	Test statistics	*p*-value	Post hoc comparisons
Age *(M, SD)*	17.3 (3.3)	17.1 (4.3)	16.4 (3.1)	$F_{2,354} = 2.04$	.131	-
Gender *(% male)*	67.6%	38.8%	48.6%	$\chi_{22,N=357} = 21.30$	<.001	ADHD>US=NC
IQ *(M, SD)*	97.8 (14.7)	101.2 (14.1)	104.5 (13.7)	$F_{2,354} = 7.29$	.001	ADHD<NC
Scan site (% Amsterdam)	40.0%	47.5%	60.7%	$\chi^2_{2,N=357} = 11.33$	.003	ADHD=US<NC
Number of ADHD symptoms *(M, SD)* [a]						
Inattentive	7.2 (1.6)	0.2 (0.6)	0.4 (0.9)	$F_{2,354} = 1427.32$	<.001	ADHD>US=NC
Hyperactive-impulsive	5.8 (2.4)	0.3 (0.7)	0.3 (0.7)	$F_{2,354} = 438.53$	<.001	ADHD>US=NC
Total	13.0 (2.9)	0.6 (1.2)	0.7 (1.4)	$F_{2,354} = 1389.21$	<.001	ADHD>US=NC
SES *(M, SD)*	11.5 (2.2)	11.4 (2.5)	13.4 (2.7)	$F_{2,345} = 23.36$	<.001	ADHD=US<NC
Medication use *(% with a history of medication use)*	90.3%	-	-			
ODD/CD *(%)*	29.4%	3.8%	0%	$\chi^2_{2,N=357} = 54.98$	<.001	ADHD>US=NC
Autism spectrum symptom score *(M, SD)*	11.9 (8.3)	3.8 (4.3)	2.0 (2.4)	$F_{2,345} = 95.55$	<.001	ADHD>US=NC

[a] Combined symptom count determined by combining scores on the K-SADS-PL and Conners' questionnaires.
Abbreviations: ADHD=Attention-Deficit/Hyperactivity Disorder; NC=normal controls; US=unaffected siblings.

Table 2. Group differences in FA and MD between ADHD, unaffected siblings, and controls.

	FA			MD		
	Coefficient (SE)	**Test statistics**	***p*-value**	**Coefficient (SE)**	**Test statistics**	***p*-value**
Fixed effects						
Age	0.088 (.026)	t_{349} = 3.372	.001	-0.157 (.040)	t_{339} = -3.87	<.001
Gender (M vs. F)	-0.038 (.180)	t_{334} = -.209	.835	-0.393 (.286)	t_{347} = -1.38	.170
Scan site (N vs. A)	6.562 (.212)	t_{234} = 30.913	<.001	11.294 (.308)	t_{194} = 36.70	<.001
IQ	-0.016 (.006)	t_{333} = -2.621	.009	-0.014 (.010)	t_{349} = -1.48	.139
Group						
NC versus ADHD	1.435 (.250)	t_{246} = 5.739	<.001	1.745 (.365)	t_{212} = 4.78	<.001
US versus ADHD	0.393 (.222)	t_{317} = 1.772	.077	1.099 (.355)	t_{335} = 3.10	.002
US versus NC	-1.040 (.275)	t_{285} = -3.796	<.001	-0.646 (.408)	t_{257} = -1.58	.114
Random effects						
Family	1.207 (.245)	Wald Z = 4.934	<.001	1.489 (.628)	Wald Z = 2.37	.018
Deviance	1756.142			2148.590		
Deviance empty model	1380.642			1692.717		

Note: indented group contrasts represent Fisher's LSD post-hoc results. FA and MD values were multiplied by 100 or 100000, respectively, for practical purposes in the analysis.
Abbreviations: ADHD=Attention-Deficit/Hyperactivity Disorder; A=Amsterdam; FA=fractional anisotropy; M=male; MD=mean diffusivity; N=Nijmegen; F=female; NC=normal controls; SE=standard error; US=unaffected siblings.

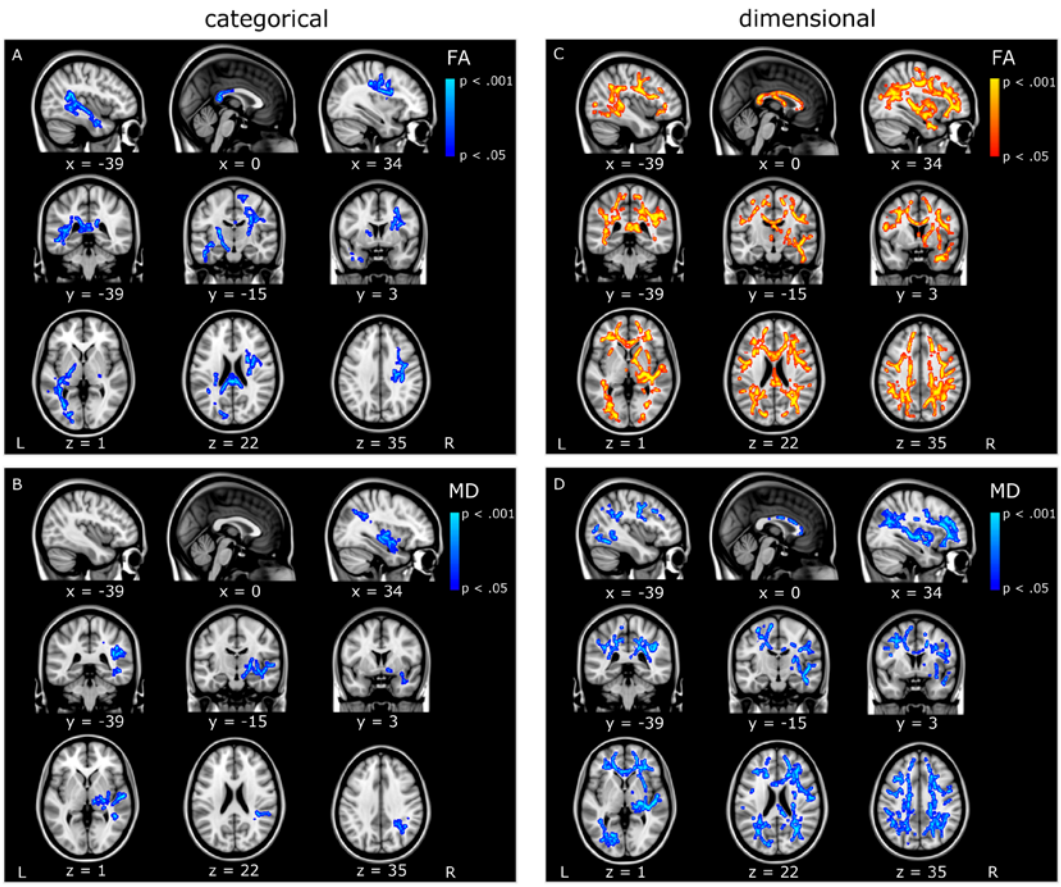

Figure 1. Results from the Tract Based Spatial Statistics (TBSS) analyses. Hot colours represent increased values for (more severe) Attention-Deficit/Hyperactivity Disorder (ADHD); cool colours represent decreased values. The left panel shows decreased fractional anisotropy (FA) (A) and mean diffusivity (MD) (B) in individuals with ADHD compared to controls. The right panel shows a positive association between ADHD symptom count and FA (C), and a negative association between ADHD symptom count and MD (D) in individuals with ADHD.

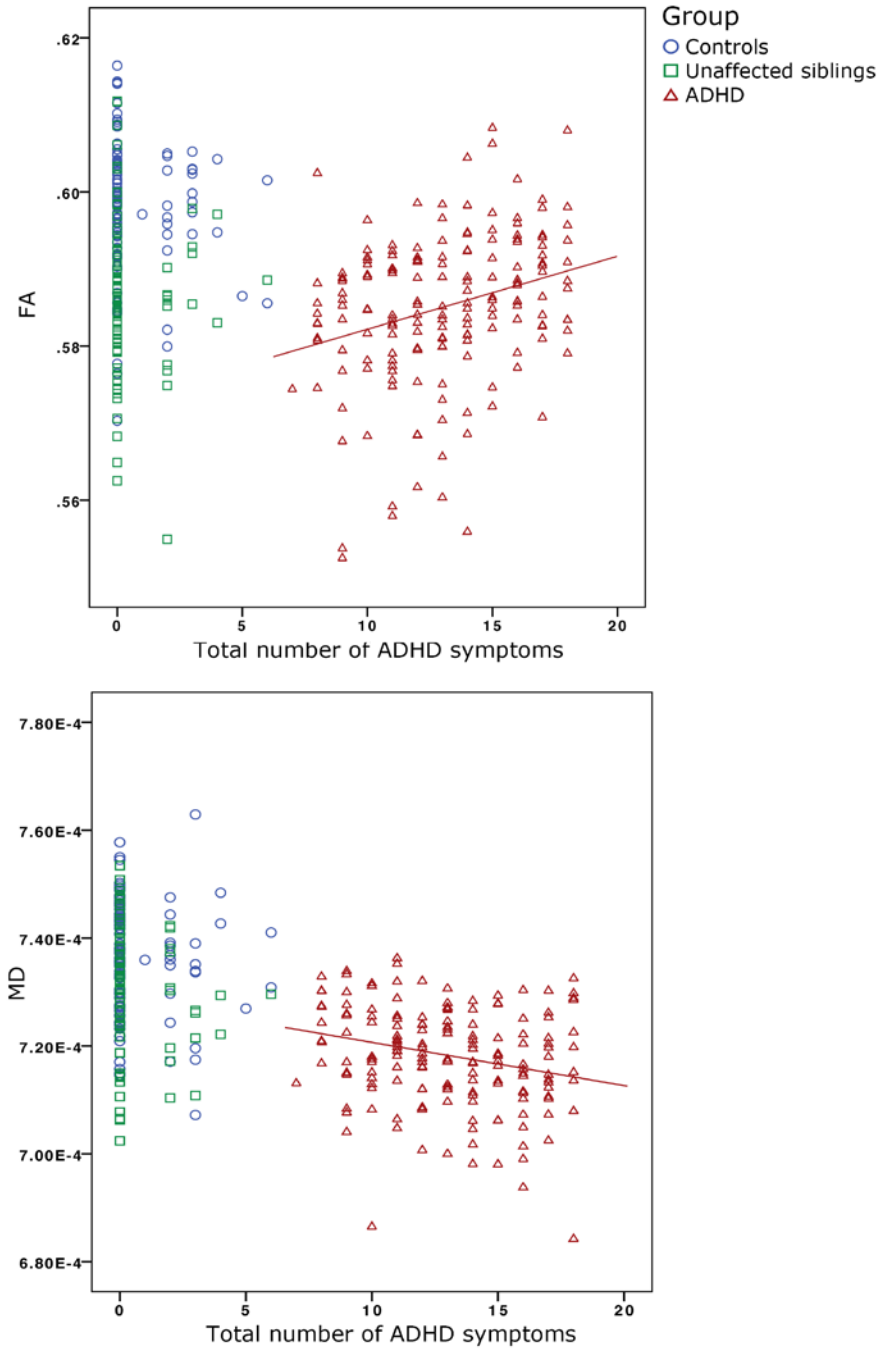

Figure 2. For illustrative purposes, mean fractional anisotropy (FA) and mean diffusivity (MD) values were plotted from overlapping voxels between the categorical and dimensional ROIs, i.e. voxels in which both the categorical and the dimensional analysis revealed significant results. The left panel shows FA values in voxels in which Attention-Deficit/Hyperactivity Disorder (ADHD) showed significantly lower FA than controls, but in which a positive association was also found between the total symptom count (inattentive and hyperactive-impulsive combined) and FA in the ADHD group. The right panel shows MD values in voxels in which ADHD showed significantly lower MD than controls, and in which a negative association was found between total symptom count and MD in the ADHD group. Note: values were plotted from all overlapping voxels; hence the shown values do not represent FA or MD in specific white matter tracts. All values are corrected for scan site, age, gender and IQ.

and age, gender or IQ. Post-hoc analyses revealed decreased FA in unaffected siblings compared to healthy controls (p<.001) and a trend towards higher FA in unaffected siblings compared to ADHD probands (p=.077). Unaffected siblings showed higher MD compared to probands (p=.002) and there were no differences in MD between unaffected siblings and healthy controls (p=.114).

A second complementary analysis was conducted to explore whether the correlation between ADHD symptom count and WM abnormalities was driven by either hyperactivity-impulsivity or inattention. Symptom counts on the two dimensions were tested as separate predictors for FA and MD in the dimensional ROI. These analyses showed a significant association with FA and MD for both inattentive and hyperactive-impulsive symptoms. Model fit significantly decreased when either one of the predictors was removed from the models, indicating that both symptom dimensions explain an important portion of the variance in FA and MD.

Finally, in each of the above complementary analyses, a set of interactions and possible confounders was tested. The ADHD predictors (either group or both symptom counts) did not interact with age, gender or IQ, and SES did not confound the results (i.e. change the relationship between the ADHD predictors and FA or MD) in the categorical and dimensional analyses. Furthermore, the presence of ODD/CD, autism spectrum score, and a history of medication use did not confound the results of the dimensional analysis.

DISCUSSION

The current study set out to investigate white matter microstructure in ADHD in a large sample of extensively phenotyped subjects with ADHD, unaffected siblings, and healthy controls, in order to obtain a robust image of WM abnormalities in ADHD. Furthermore, we aimed to investigate the association between ADHD symptom count and WM pathology, and explore the familiality of WM abnormalities. A widespread pattern of disturbed white matter microstructure was found in individuals with ADHD compared to healthy controls, consistent with the previous literature (Cao et al., 2010; Cortese et al., 2013; Dramsdahl et al., 2012; Van Ewijk et al., 2012). Clusters of decreased FA and MD were associated with ADHD in most of

the major WM tracts. Dimensional analyses revealed a strong and widespread association between ADHD symptom count and WM abnormalities in ADHD patients, represented by higher FA and lower MD at higher symptom levels, showing an overlap of only 25% with areas that showed disturbances in the categorical analyses. The association was present for inattentive as well as hyperactive-impulsive symptoms, suggesting that both symptom dimensions play a role in WM pathology in ADHD. The association between ADHD and FA and MD did not interact with gender or IQ, indicating that WM pathology is present in both males and females with ADHD and at all levels of cognitive functioning. Furthermore, the association between ADHD and WM pathology did not interact with age (see Supplementary Figure S1) and our main findings were largely replicated in children, adolescents and young adults separately (see Supplement 1), suggesting that WM pathology in ADHD does not ameliorate with age. Results were not confounded by the presence of comorbid ODD/CD, subclinical ASD symptoms, a history of medication treatment, or socio-economic status. Clusters of abnormal FA and MD were located in widespread regions throughout the brain. The widespread nature of WM deficits is consistent with findings of other neuroimaging studies in which abnormalities have been found in all lobes of the brain (Cortese et al., 2012), as well as studies into neurocognitive deficits in ADHD, in which a wide range of cognitive deficits is observed (Willcutt et al., 2005). Altogether, these findings may implicate a general deficit in brain structure and functioning, rather than a local abnormality.

Unaffected siblings showed decreased FA similar to ADHD probands, which is in part consistent with a previous study, which reported the first evidence for WM abnormalities in unaffected siblings (Lawrence et al., 2013). However, while the study by Lawrence and colleagues showed increased MD in unaffected siblings compared to controls, we found decreased FA in unaffected siblings compared to controls, but no abnormalities in MD. It has to be noted that the analyses in our study differ from those conducted by Lawrence and colleagues, which hampers the comparability of results. While Lawrence and colleagues investigated MD in the forceps minor, we investigated FA and MD in several regions that showed group differences between ADHD and controls, but these regions did not include the forceps minor. Importantly, while FA in unaffected siblings was significantly lower than in healthy controls, a trend was also observed towards higher FA compared to ADHD probands. This pattern, in which unaffected siblings seem to be intermediate between individuals with ADHD and controls, suggests that WM pathology, as reflected by decreased FA, indexes a familial shared genetic vulnerability for the disorder.

Importantly, our analyses show results that may seem inconsistent at first glance, but could be explained by separate mechanisms of WM abnormalities in ADHD. First, clusters of decreased FA and MD were located in different, non-overlapping regions. Second, while both measures were associated with behavioural symptoms of the disorder, FA, but not MD, was linked to a familial vulnerability to the disorder. These discrepancies suggest a fundamental difference between the decreases in FA and MD we found. Third, findings from categorical and dimensional analyses were not consistently located in the same brain regions, and dimensional findings were more widespread than categorical findings. For MD, this may illustrate that dimensional analyses have more power to detect an association between ADHD and WM pathology. For FA, however, findings did not only differ in terms of location, but were also in opposing directions. This seems to implicate two fundamentally different types of WM pathology underlying FA alterations in ADHD.

Integrating these findings, we hypothesize that two different, separate mechanisms underlie WM abnormalities in ADHD, and that these mechanisms are possibly driven by different factors and may correspond to different types of WM pathology on a neurobiological level. A first mechanism (Figure 1A) is characterized by decreased FA in individuals with ADHD as well as in unaffected siblings, and is not (dimensionally) associated with ADHD symptom count. Therefore, this mechanism appears to be linked to a familial vulnerability to the disorder, rather than the behavioural symptoms. That is, individuals with a familial (genetic or common environmental) risk for ADHD have a vulnerability to WM disturbances - as represented by decreased FA. For example, ADHD risk genes could cause poor myelination in individuals with ADHD and their family members. A second mechanism is characterized by a widespread association between ADHD symptom count and WM abnormalities (represented by lower MD and higher FA; Figure 1B, 1C, and 1D). MD values in unaffected siblings were comparable to those of control subjects, and no elevated FA was observed in unaffected siblings. Therefore, this second mechanism appears to be linked to the clinical state (i.e. the presence of behavioural symptoms) rather than a familial vulnerability. For example, environmental risk factors for ADHD (such as pre- or perinatal difficulties) or the presence of symptoms in itself could cause decreased axonal density or neurite outgrowth in individuals with ADHD.

The presence of such different mechanisms may play an important role in the heterogeneity of findings in the current literature. Our findings suggest that, depending on the number of symptoms of the ADHD patients and which brain regions are investigated, different patterns

of WM disturbances can be found in ADHD. For FA, we showed that it is possible to find lower FA (compared to controls) as well as higher FA (in patients with a higher number of symptoms), depending on the analysis and the number of ADHD symptoms. Moreover, disturbances in MD were strongly associated with ADHD symptom count, suggesting that studies including more severe patients are more likely to find group differences in MD. Therefore, it is important for future studies not to limit their analyses to group differences, but also investigate the influence of symptom count on parameters of WM microstructure.

It is important to note that the interpretation of abnormal DTI parameters in psychiatric disorders remains somewhat speculative (Alexander et al., 2007; Jones et al., 2013). For example, in a single fibre bundle, decreased FA may represent myelin breakdown, while in regions with crossing fibres it may represent increased neuronal branching, and could as such even indicate increased structural connectivity. Hence, it is difficult to interpret our findings of abnormal FA and MD in terms of more specific neurobiological mechanisms. Furthermore, heterogeneity in sample characteristics and research methods in the current literature is substantial (including, but not limited to, age range, inclusion of comorbidities, DTI parameters and analysis methods, and statistical corrections; Van Ewijk et al., 2012). Altogether, inconsistencies between studies and a lack of a straightforward interpretation of analysis parameters currently restrict a more elaborate interpretation of WM abnormalities in general, and in ADHD specifically. These limitations should be kept in mind while interpreting not only our findings, but also the current DTI literature in psychiatric disorders as a whole.

Taken together, our results implicate separate patterns of disturbed WM microstructure in ADHD. A first mechanism is characterized by decreases in FA in ADHD patients as well as their unaffected siblings, which may be linked to a familial vulnerability to the disorder. A second, separate mechanism is characterized by an association between ADHD symptom count and WM abnormalities (represented by lower MD and higher FA), and appears to be more closely linked to the clinical state, i.e. the presence of behavioural symptoms of the disorder. It is likely that different factors underlie these mechanisms (e.g. risk genes versus environmental risk factors), and that they result in different types of pathology (e.g. disturbed myelination versus axonal density). Such different mechanisms may explain an important portion of heterogeneity in the current literature on WM pathology in ADHD, and may also play a role in other psychiatric disorders.

SUPPLEMENT 1

Supplementary analyses: Age

To test whether our main results applied to the full age range of our sample, we created 3 age groups: children (8-13 years; 29 ADHD, 23 controls), adolescents (14-18 years; 88 ADHD, 59 controls), and young adults (19-30 years; 53 ADHD, 25 controls). Multilevel models were built, similar to all other follow-up analyses, to replicate our main results in the three age groups. In the categorical ROI, FA and MD was compared between ADHD and controls, while controlling for age, gender, scan site and IQ. In the dimensional ROI, the association between ADHD symptom count and FA and MD was investigated, using the same covariates. Furthermore, for visualisation purposes, predicted FA and MD values from the categorical analysis were plotted over the full age range (Figure S1).

Results were similar to those of the main TBSS analyses in all age groups, i.e. decreased FA and MD in ADHD compared to controls, and an association between higher symptom count and higher FA and lower MD. However, two of the results were no longer significant: categorical group differences on MD in the young adult group (p=.106) and the dimensional association between symptom count and MD in the children group (p=.211). The lack of significance of these two models may be due to a lack of power, given that the youngest and oldest age group had a relatively low number of participants.

In conclusion, our main findings could largely be replicated in all age groups (children, adolescents and young adults), except for two results: decreased MD in young adults with ADHD, and the association between symptom count and MD in children. The absence of these two findings could implicate a developmental aspect of MD abnormalities in ADHD, but may also be due to a lack of power in these two age groups. In our main analyses, age did not interact with group or symptom count, and plotted FA and MD values from our categorical analysis (Figure S1) do not show evidence of differences in white matter pathology across different ages. Consequently, taken together, our results do not implicate differences in WM pathology in ADHD throughout the development. However, it should be noted that the majority of participants in our sample were adolescents. As such, developmental effects could not be thoroughly investigated in the current sample. Results need to be replicated in bigger samples of children and (young) adults to investigate whether our findings generalize to all ages.

SUPPLEMENTARY FIGURE

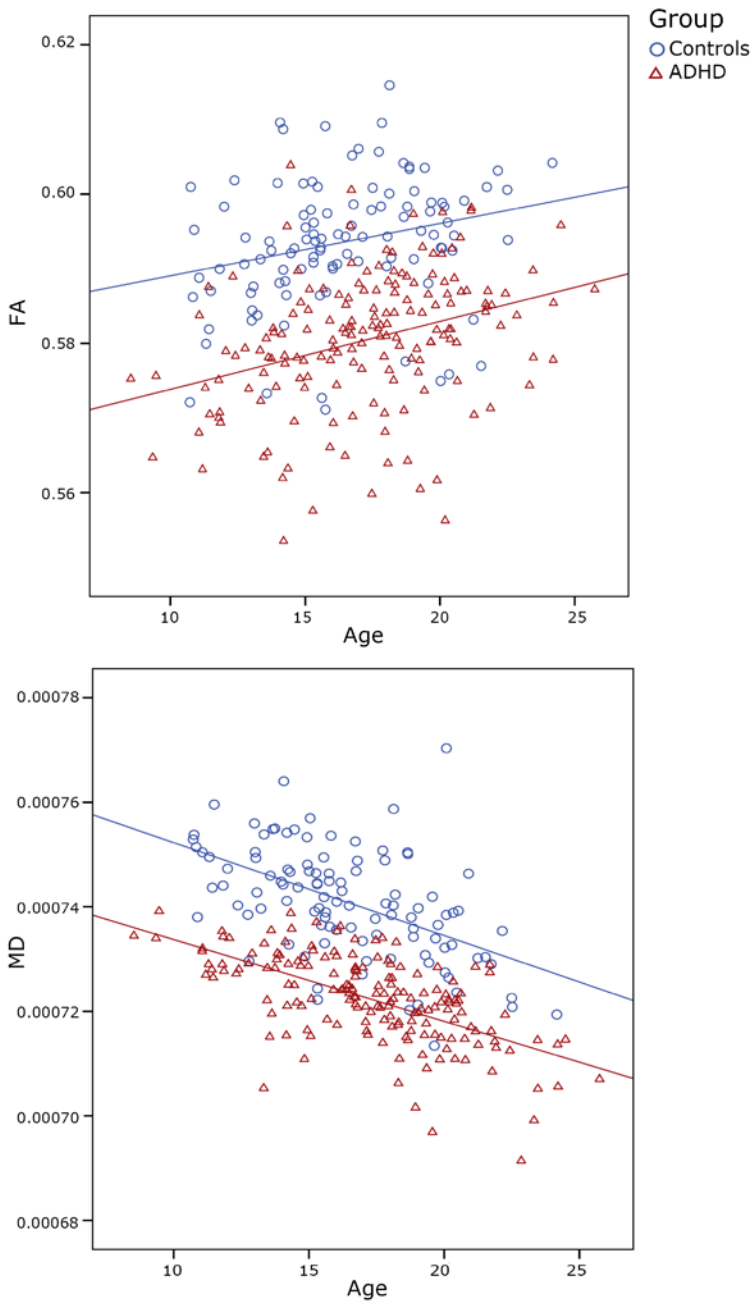

Figure S1. For illustrative purposes, mean fractional anisotropy (FA) and mean diffusivity (MD) values were plotted from voxels showing a significant result in the categorical TBSS analysis. The figure shows lower FA and MD in Attention-Deficit/Hyperactivity Disorder (ADHD) compared to controls, and a linear association between age and FA and MD. No significant interaction was found between group and age on either FA or MD, suggesting that white matter pathology in ADHD is stable and persists into young adulthood. Note: values were plotted from *all* significant voxels; hence the shown values do not represent FA or MD in specific white matter tracts. All values are corrected for scan site, age, gender and IQ, and for display purposes, predicted values were demeaned for scan site.

SUPPLEMENTARY TABLE

Table S1. Clusters of significant results from the TBSS analyses.

Analysis		Cluster	Anatomical label(s)	Hemisphere	n Voxels	MNI coordinates			p-value
						x	y	z	
Categorical	**FA**	1	Anterior thalamic radiation, corticospinal tract, ILF, internal capsule, SLF	Left	3824	-32	-32	4	.015
		2	Corticospinal tract, inferior FOF, internal capsule, SLF	Right	2626	36	0	32	.028
		3	Corpus callosum (splenium, isthmus), forceps major	Bilateral	1066	-1	-33	21	.034
		4	Temporal WM	Left	566	-41	-2	-28	.040
		5	Inferior FOF, SLF	Right	264	30	8	13	.044
		6	Temporal WM	Left	103	-28	1	-34	.045
		7	Corpus callosum (body)	Bilateral	21	14	-32	28	.049
	MD	1	Corticospinal tract, ILF, inferior FOF, SLF	Right	3536	41	-30	-15	.030
		2	Inferior FOF	Right	402	9	11	-15	.043
Dimensional	**FA**	1	Anterior corona radiata, Anterior/posterior thalamic radiation, corticospinal tract, forceps major, forceps minor, inferior FOF, ILF, internal capsule, SLF	Right	22827	37	-32	7	.014
		2	Anterior corona radiata, Anterior/posterior thalamic radiation, corticospinal tract, forceps major, forceps minor, inferior FOF, ILF, SLF	Left	14280	-41	-1	23	.019
		3	ILF	Right	22	39	-48	-5	.047
	MD	1	Anterior corona radiata, anterior thalamic radiation, corpus callosum (splenium, body, genu), forceps minor Forceps major, posterior corona radiata SLF	Bilateral Left Right	13188	33	34	-2	.016
		2	Anterior corona radiata, corpus callosum (splenium, body, genu), cingulum, corticospinal tract, inferior FOF, internal capsule	Right	6490	35	-24	-2	.021
		3	Corpus callosum (splenium, body) Corticospinal tract, Internal capsule, ILF, posterior/superior corona radiata,	Bilateral Right	782	-36	-7	40	.041

SUPPLEMENTARY TABLE (CONTINUED)

Analysis	Cluster	Anatomical label(s)	Hemisphere	n Voxels	MNI coordinates			p-value
					x	y	z	
	4	SLF	Right	344	45	9	10	.048
	5	ILF	Left	250	-37	-73	-6	.045
	6	SLF	Right	205	56	-12	21	.047
	7	Parietal WM	Left	199	-33	20	34	.048
	8	ILF	Left	172	-37	-50	-6	.045
	9	Forceps major, ILF	Left	140	-19	-69	0	.047
	10	ILF	Left	122	-20	-82	31	.048
	11	SLF	Right	106	35	0	32	.049
	12	Parietal WM	Left	86	-53	-31	30	.048
	13	Forceps Major	Left	66	-27	-85	-4	.049
	14	ILF, inferior FOF	Left	61	-13	-83	-8	.049
	15	Inferior FOF	Left	50	-30	-70	1	.049
	16	SLF	Right	34	47	-3	44	.048
	17	SLF	Right	29	47	-1	33	.049

Note: Categorical results represent decreased FA and MD in ADHD compared to controls, dimensional results represent a positive association between ADHD severity and FA, and a negative association between ADHD severity and MD. The table shows significant clusters at $p<.05$ (corrected for multiple comparisons), controlling for scan centre, age, IQ and gender. MNI coordinates represent maximum intensity voxel.
Abbreviations: FA=fractional anisotropy; FOF=fronto-occipital fasciculus; MD=mean diffusivity; ILF=inferior longitudinal fasciculus; SLF=superior longitudinal fasciculus; TBSS=tract based spatial statistics; WM=white matter.

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